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Depuration of microcystin-LR from the red swamp crayfish *Procambarus clarkii* with assessment of its food quality

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ABSTRACT

In the latest years, blooms of toxic Cyanobacteria have intensified worldwide, causing serious problems to human health. The edible crayfish *Procambarus clarkii* is known to accumulate the toxins (microcystins) produced by *Microcystis aeruginosa*. Our aims here were to (1) quantify microcystin-LR in *P. clarkii*'s organs/tissues; (2) analyse differences in its content between size classes and between sexes; (3) develop protocols for the depuration of the toxin from crayfish; and (4) assess the quality of the crayfish abdominal muscle during depuration. The study was composed of two phases. In the first, the concentration of microcystin-LR in the hepatopancreas, stomach, intestine, and abdominal muscle of 102 crayfish was measured by the means of ELISA and was compared between small and large individuals and between sexes. In the second phase, 136 crayfish (64 males and 64 females) were subject to depuration for a maximum period of 21 days under two feeding regimes (*ad libitum*, i.e. fed every day or restricted, i.e. once every third day). Sixteen crayfish were sacrificed at different times from the start of the treatment (3, 6, 9, 14, 17, and 21 days). Microcystin-LR was measured in the intestines and in the abdominal muscles of the treated crayfish and was compared to the values obtained from individuals sacrificed immediately upon capture. The abdominal muscle was also analysed for its quality by quantifying colour, water holding capacity, and composition (qualitative and quantitative) of the total lipids and fatty acids. The results showed that (1) *P. clarkii* accumulates more toxin in the intestine and less in the abdominal muscle (the edible part); (2) microcystin-LR is less abundant in large (the marketable size) than in small individuals; and (3) its content differs between sexes in the hepatopancreas only. Besides, (4) microcystin-LR content decreased in the abdominal muscle, but not in the intestine, after a short period of depuration in the two feeding regimes and (5) depuration did not reduce the overall quality of crayfish meat.

1. Introduction

Cyanobacteria form dense blooms in nutrient enriched waters. In the latest years, the frequency and intensity of these blooms have increased due to the growing water eutrophication, with consequent threats to the ecology of the affected waterbodies and to human health (Carmichael and Falconer, 1993; Cox et al., 2005). Over 40 species of Cyanobacteria are known to produce toxins in given conditions (e.g. at high pH and water temperature) (Carmichael, 1997). Exposure to these toxins can either directly kill organisms or decrease their resistance to bacterial or viral infections (Carmichael, 1996). Microcystins, produced by several genera of Cyanobacteria, such as *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria*, are considered to be one of the most dangerous groups of cyanotoxins (Carmichael, 1997). However, among the toxic Cyanobacteria genera, *Microcystis aeruginosa* continues to be the most commonly reported (Park et al., 2001). This species lives in relatively quiet waters and forms surface water blooms between summer and autumn, possibly favoured by the elevated irradiation (Krishnamurthy et al., 1986).

Microcystins are cyclic heptapeptides. To date, over 60 variants of microcystins have been identified, but microcystin-LR is the most toxic (Sivonen and Jones, 1999). It has diversified effects on species (Hansson et al., 2007); in fact, animal sensitivity highly changes with the organism, the variant, and the mode of exposure (Codd and Poon, 1998). Microcystins are hepatotoxins, known to harm human liver (the principal target), lungs, and kidneys (Krishnamurthy et al., 1986); they may act as potent tumour promoters (Falconer, 1991), their accumulation being associated with an increased incidence of liver cancer (Dunn, 1996; Fujiki and Suganuma, 1993). Consumption of toxin-contaminated water has been implicated in the loss of livestock and wild animals in several countries, as well as in human intoxication (Krishnamurthy et al., 1986). In 1998, the World Health Organization (WHO) set the Tolerable Daily Intake (TDI) for human ingestion of

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microcystin-LR at 0.04 μg kg^{-1} body weight per day and the limit of its concentration in the water at 1 μg L^{-1} (or 5000 cells mL^{-1} as set by the Italian Ministry of Health in the Ministerial Memorandum on Bathing Waters, July 31, 2008.

In the last decade, blooms of *M. aeruginosa*, leading to the formation of dense "scums", have been observed in Massaciuccoli Lake, a large wetland between Pisa and Lucca (Tuscany, Italy). This area is also characterised by the presence, since the 1990s, of a naturalised population of the red swamp crayfish *Procambarus clarkii* (Decapoda, Cambaridae). *P. clarkii*, native to north-eastern Mexico and south-central USA, has been imported into many countries mainly for aquaculture purposes (Huner, 2002; Gherardi, 2006). Today it is the most diffused and invasive freshwater crayfish in the world (Gherardi and Holdich, 1999). Its plastic life cycle, high fecundity, and tolerance to extreme conditions make its cultivation easy, but also favour its spread in the wild (Gherardi, 2006). Crayfishing may contain the diffusion of this species and certainly contributes in mitigating its ecological damages, also helping the local economy (e.g. in Spain; Ackefors, 2000). This practise has developed in Massaciuccoli Lake since 1997 (Cenni, 1997), but, due to the cyanobacterial blooms occurring in the summer (when crayfishing is mostly practised), it has been temporarily banned (in 2002 and 2003; Simoni et al., 2004) with economic losses for local fishermen. Previous studies have shown that this and other crayfish species feed on pelagic/surface microalgae, including Cyanobacteria (Gherardi and Lazzara, 2006), and accumulate toxins in their tissues (Lirås et al., 1998; Vasconcelos et al., 2001; Simoni et al., 2004), without showing any apparent change in their behaviour or vitality (Lirås et al., 1998). Notwithstanding this, no protocols are available to date to assess the safety of crayfish meat for the consumers.

The present study aimed to (1) quantify microcystin-LR in different organs/tissues of *P. clarkii*; (2) analyse differences in its content between small and large individuals and between sexes; (3) develop protocols for the depuration of the toxin from crayfish; and (4) assess the quality of the crayfish abdominal muscle during depuration.

2. Methods

2.1. Experimental design

The study was composed of two phases: in the first, we investigated whether sexes and size classes differed for the content of microcystin-LR (hereafter indicated as "microcystin") in the different organs/tissues; in the second phase we developed a protocol for crayfish depuration that might maintain the quality of the edible part (abdominal muscles).

2.2. Differences between sexes and size classes

In October 2005, when the mean concentration of microcystin in the water, as assessed by ELISA, was 1.76 μg L^{-1}, 102 crayfish were collected with traps in Massaciuccoli Lake (90 km from Florence, Italy). The animals were immediately frozen. In the laboratory, each crayfish was weighed with an electronic balance (accuracy: 1 mg); its cephalotorax length (CL, from the tip of the rostrum to the posterior edge of the carapace) was measured with a vernier caliper (accuracy: 0.05 mm), and its sex was determined. Based on their CL (ranging between 33 and 101 mm), crayfish were classified into small (CL<35 mm) or large (CL≥35 mm) individuals. The hepatopancreas, stomach, intestine, and abdominal muscle of each of them were removed and weighed as above.

2.3. Toxin extraction and analysis

Microcystin was extracted following the method by Krishnamurthy et al. (1986), modified by Magalhães et al. (2001, 2003). After several extractions with methanol and hexane, the analysis was done by the means of ELISA using an EnviroGard® Microcystins Plate Kit (Strategic Diagnostic Inc.). The assay uses antibodies against microcystin-LR (MCYST-LR). This method has a sensitivity of 0.1 ng MCYST mL^{-1}. As suggested by Metcalf et al. (2000), ELISA can also detect microcystin conjugates of lower toxicity, thus overestimating the potential toxicity (in terms of microcystin-LR content) of the analysed samples. However, it is still regarded as one of the most reliable methods for the analysis of this toxin (Metcalf et al., 2000).

Each crayfish provided multiple organs/tissues for the analysis. The analysis of microcystin content was done on samples of similar fresh weight (1 g for intestine and stomach, 3 g for hepatopancreas and abdomen) obtained by pooling the organs/tissues of 6 crayfish (total 36 males and 66 males, or 41 small and 61 large crayfish).

2.4. Depuration

In September 2006, when the mean concentration of microcystin in the water, as assessed by ELISA, was 0.20 μg L^{-1}, 136 large crayfish (68 males and 68 females; CL≥35 mm) were collected in Massaciuccoli Lake as above. In the laboratory, 4 males and 4 females (day 0) were immediately sacrificed. The other individuals were assigned to one of four plastic tanks (112.5×112.5×75.5 cm; water depth: 30 cm; crayfish density: 0.14 m^{-2}) provided with refuges and maintained, for a maximum of 21 days, in a temperature-controlled room (15.62±0.39 °C) under a natural 14:10 light:dark cycle. Tanks, each corresponding to a treatment, differed for the sex of the hosted crayfish and for the feeding regime to which they were subject, i.e. every day (*ad libitum*) or once every third day (restricted). Crayfish were fed with commercial pellets (crude protein: 41.0%, crude fat: 12.0%) and corn seeds. Thirty-four crayfish died during the depuration period.

Four crayfish were randomly collected from each tank at day 3, 6, 9, 14, 17, and 21 from the start of the treatment. They were killed by hypothermia; each of them was weighed with an electronic balance to the nearest 1 mg (mean±SE: 15.86±6.87 g), and their total body length (mean±SE: 83.44±12.55 mm) and CL (mean±SE: 42.36±6.95 mm) were measured with a vernier caliper (accuracy: 0.05 mm). The intestine and the abdominal muscle were removed and weighed; the content of microcystin was analysed as above.

2.5. Assessment of food quality

The measures taken as indicators of the quality of the abdominal muscle were as follows:

1) yield, the percentage of edible muscle in relation to body weight;
2) colour parameters (L^* or lightness, a^* or red/green chromaticity index, b^* or yellow/blue chromaticity index, according to the CIELab system; CIE, 1976), measured with a Chroma-Meter Minolta (Mod. CR-200) on the dorsal and ventral sides;
3) water holding capacity, measured in a sample of 300 mg as free water by compression method (Grau and Hamm, 1957);
4) moisture (AOAC, 1995), total lipids (Folch et al., 1957), and qualitative and quantitative (using the C23:0 as internal standard) composition of fatty acids (Morrison and Smith, 1964). Quantitative data of the fatty acids were used to calculate the atherogenicity (AI) and the trombogenicity (TI) indices, according to Ulbricht and Southgate (1991), as follows:

$$AI = [C12{:}0 + (4{*}C14{:}0) + C16{:}0]/(\sum MUFA + \sum PUFAn6 + \sum PUFAn3)$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5{*}\sum PUFAn6) + (3{*}\sum PUFAn3) + (0.5\sum MUFA) + (\sum PUFAn3/\sum PUFAn6)]$$

2.6. Data analyses

We followed the procedures suggested by Siegel and Castellan (1988). Independent samples were analysed by Mann–Whitney *U*-

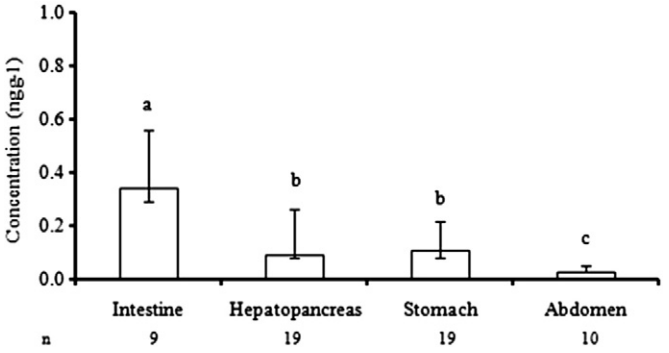

Fig. 1. Concentration of microcystin (medians and interquartile ranges) in the analysed tissues/organs (fresh weight). Letters over bars denote the hierarchy after Multiple Comparisons tests. *n* indicates the number of pools per tissue/organ on which the analysis was done; each pool (of approximately the same weight) was composed of the tissues/organs from 102 crayfish.

tests (statistic: *U*) and Kruskal–Wallis analyses of variance (statistic: *H*), and dependent samples by Friedman two-way analyses of variance (statistic: *Fr*). If differences among samples were significant after Kruskal–Wallis and Friedman analyses, Multiple Comparisons tests were applied to determine which pairs differed significantly.

The data related to the food quality assessment were analysed by three-way ANOVAs (factors: sex, feeding regime and days of depuration; statistic: *F*), using the PROC GLM of SAS® Package (SAS, 1997). The interactions sex×days of depuration and feeding regime×days of depuration were also tested.

Figures give medians and interquartile ranges (first-third quartiles) or means and SE. Tables give means and residual standard deviations (r.s.d.). The level of significance at which the null hypothesis was rejected is $\alpha = 0.05$.

3. Results

3.1. Differences between sexes and between size classes in microcystin content

Microcystin was found in all the organs/tissues analysed, mostly in the intestine (Fr = 18.60, $df = 3$, $P < 0.001$; after Multiple Comparison Test: intestine > hepatopancreas = stomach > abdominal muscle) (Fig. 1). No differences were found between sexes (*U* between 6.5 and 36, *n* between 3 and 12, *P* between 0.61 and 0.81), except in the hepatopancreas (females > males; $U = 17.5$, $P = 0.03$, males: $n = 8$, median = 0.08 ng g^{-1}, females: $n = 12$, median = 0.27 ng g^{-1}). Microcystin content was significantly higher in small than in large crayfish in all the organs/tissues analysed (intestine: $U = 0.00$, $P = 0.04$; hepatopancreas: $U = 17.5$, $P = 0.03$; stomach: $U = 0.00$, $P < 0.0001$; abdominal muscle: $U = 0.00$, $P = 0.02$) (Fig. 2).

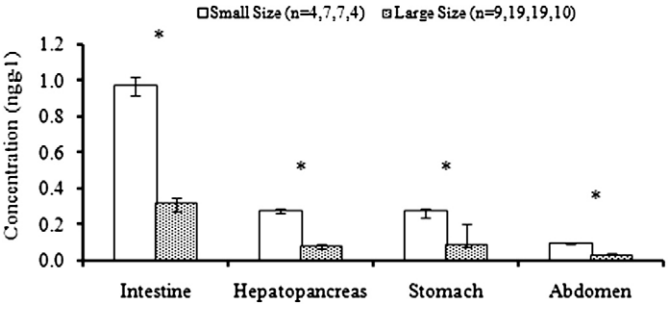

Fig. 2. Comparisons between size classes for the concentration of microcystin (medians and interquartile ranges) in the analysed tissues/organs (fresh weight). Asterisks over bars denote significant differences after Mann–Whitney *U*-tests. *n* indicates the number of pools per tissue/organ on which the analysis was done; each pool (of approximately the same weight) was composed of the tissues/organs from 61 large and 41 small crayfish.

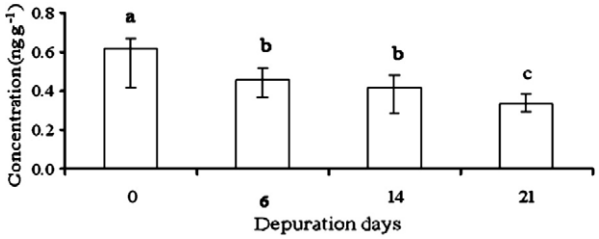

Fig. 3. Concentration of microcystin (medians and interquartile ranges) in the abdominal muscle (fresh weight) during the depuration period. Letters over bars denote the hierarchy after Multiple Comparisons tests.

3.2. Depuration

Microcystin content significantly decreased with time in the abdominal muscle ($H = 10.24$, $df = 3$, $P < 0.05$; after Multiple Comparison Test: day 0 > day 6 = day 14 > day 21) (Fig. 3), without any difference either between sexes (Table 1a) or between feeding regimes (Table 1a). On the contrary, it remained nearly constant in the intestine ($H = 3.46$, $df = 3$, $P = 0.33$; median concentration between 19.61 and 22.24 ng g^{-1}) in either sex (Table 1b) or feeding regime (Table 1b).

Overall sexes did not differ for yield, *L**, and chromaticity indices. The only intersexual difference was found for *a** of the ventral side of the abdomen (females > males) (Table 2). Similarly, the two feeding regimes had no effect on yield and colour parameters. On the contrary,

Table 1
Comparisons between sexes and feeding regimes during the depuration period for the concentration of microcystin in the abdominal muscle (A) and in the intestine (B), using Mann–Whitney *U*-tests (statistic: *U*)

Day	Sex			Feeding regime		
	U	*n*	*P*	*U*	*n*	*P*
A						
0	4	8	0.48	–	–	–
6	4	13	0.48	8	13	0.12
14	8	14	0.41	11	14	0.17
21	9	15	0.73	16	15	1.00
B						
0	4	8	0.25	–	–	–
6	17	13	0.94	16	13	1.00
14	7	14	0.09	18	14	1.00
21	16	15	0.81	4	15	**0.03**

Significant differences are shown in bold.

Table 2
Colour parameters of the abdominal muscle, at dorsal and ventral sides

	Sex		Feeding regime (FR)		Depuration days (DD)	Sex* (DD)	FR* (DD)	r.s.d.
	♀ (*n* = 52)	♂ (*n* = 42)	*Ad libitum* (*n* = 50)	Restricted (*n* = 44)				
Abdominal muscle (%)	12.27	12.70	12.31	12.67	n.s.	n.s.	n.s.	3.65
Colour (dorsal)								
*L**	48.18	49.47	48.88	48.77	***	n.s.	n.s.	6.47
*a**	5.44	4.60	4.95	5.10	***	*	*	2.77
*b**	4.31	3.61	4.19	3.73	***	n.s.	n.s.	3.40
Colour (ventral)								
*L**	46.31	47.92	46.47	47.76	***	n.s.	n.s.	6.42
*a**	4.21	3.25	3.76	3.71	***	**	**	2.32
*b**	2.26	1.90	2.46	1.71	***	n.s.	n.s.	2.97

*** and ** denote $P < 0.001$ and $P < 0.01$, respectively.
n.s.: not significant.

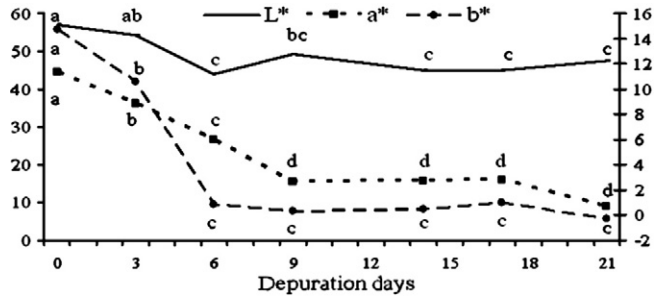

Fig. 4. Changes in the colour parameters L^* (left), a^* and b^* (right), measured at the dorsal side of the abdominal muscle, during the depuration period. Letters over bars denote the hierarchy after Multiple Comparisons tests.

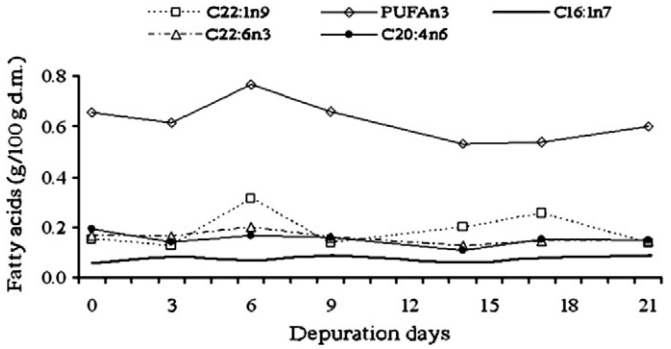

Fig. 5. Changes in the content of PUFAn3, C16:1n7 C22:1n9, C22:6n3 and C20:4n6 of the abdominal muscle during the depuration period.

the latter changed with time (Table 2, Fig. 4): the abdominal muscle became darker since day 6 and the redness and yellowness indices decreased since day 1. After day 6, all the colour parameters showed a significant change.

Water holding capacity varied with time (data not reported) without any difference between sexes and feeding regimes. A significant increase was found in the muscle of males after day 14 (4.66 cm^2 until day 9; 9.68, 6.55, and 15.65 cm^2 at days 14, 17, and 21, respectively; day 3 = day 6 = day 9 < day 14 = day 17 < day 21; $P = 0.006$). Sex and feeding regime had no effect on either moisture or the total lipid content (about 4% of dry matter; Table 3). Differences between sexes in fatty acid content were not significant, except for C16:1n7 and ΣPUFAn4 (females > males) and for C22:1n9 and C22:6n3 (males > females). Moisture increased with time (from 74.22% at day 0 to 81.44% at day 21), whereas total lipids and some single fatty acids did not show a clear pattern of change (Table 3 and Fig. 5). On the contrary, C22:6n3, ΣPUFAn3 (day 6 > day 0 = day 3 = day 9 > day 14 = day 17 > day 21), and C20:4n6 (day 0 > day 3 = day 6 = day 9 = day 17 = day 21 = > day 14) significantly decreased (Table 3 and Fig. 5). The percentage of ΣMUFA on the total of fatty acids was lower in males than in females, whereas the percentages of ΣSFA, C16:0, C17:0, and C22:6n3 were higher (Table 4).

The fatty acid profile was not altered by the feeding regime with a few exceptions (Table 4) but it changed with time, with ΣSFA, C17:0, and C20:4n6 decreasing, and ΣMUFA, C16:1n7, and C18:2n6 increasing. On the contrary, PUFAn6, EPA, PUFAn3/PUFAn6 ratio, and the atherogenicity and trombogenicity indices remained constant.

Table 3
Total lipid content and fatty acid composition in the abdominal muscle

	Sex		Feeding regime (FR)		Depuration days (DD)	Sex * (DD)	FR * (DD)	r.s.d.
	♀	♂	*Ad libitum*	Restricted				
Moisture (%)	80.28	79.73	79.97	80.05	***	n.s.	n.s.	2.80
Total lipids (% d.m.)	4.23	4.31	4.29	4.25	*	n.s.	n.s.	0.73
FA. (g/100 g d.m.)								
C16:0	0.29	0.32	0.30	0.30	n.s.	n.s.	n.s.	0.06
C18:0	0.16	0.17	0.16	0.16	n.s.	n.s.	n.s.	0.04
ΣSFA	0.51	0.55	0.53	0.53	n.s.	n.s.	n.s.	0.11
C16:1n7	0.09^{A}	0.06^{B}	0.07	0.07	*	**	n.s.	0.02
C18:1n9	0.51	0.51	0.51	0.51	n.s.	n.s.	n.s.	0.12
C22:1n9	0.16^{b}	0.21^{a}	0.20	0.18	***	n.s.	n.s.	0.07
ΣMUFA	0.66	0.62	0.64	0.63	n.s.	n.s.	n.s.	0.14
Σ Not identified F.A.	0.05	0.06	0.06	0.05	n.s.	n.s.	n.s.	0.02
ΣPUFAn4	0.0012^{a}	0.0008^{b}	0.001	0.0009	**	n.s.	n.s.	0.00
C18:3n3	0.09	0.09	0.09	0.08	n.s.	n.s.	n.s.	0.03
C20:5n3	0.35	0.36	0.35	0.35	n.s.	n.s.	n.s.	0.08
C22:6n3	0.14^{B}	0.18^{A}	0.16	0.15	*	n.s.	n.s.	0.04
ΣPUFAn3	0.60	0.65	0.64	0.61	*	n.s.	n.s.	0.12
C18:2n6cis	0.15	0.14	0.15	0.14	n.s.	n.s.	n.s.	0.05
C20:4n6	0.15	0.16	0.16	0.14	**	n.s.	n.s.	0.03
ΣPUFAn6	0.40	0.35	0.36	0.33	n.s.	n.s.	n.s.	0.07
PUFAn3/PUFAn6	1.84	1.93	1.77	2.00	n.s.	n.s.	n.s.	0.40
Atherogenicity index	0.21	0.22	0.21	0.22	n.s.	n.s.	n.s.	0.02
Thrombogenicity index	0.11	0.11	0.11	0.11	n.s.	n.s.	n.s.	0.03

F.A. = fatty acids; d.m. = dry matter; r.s.d. = residual standard deviation.
Superscripts denote significant differences (A, B: for $P<0.01$; a, b: for $P<0.05$).
***, **, and * denote $P<0.001$, $P<0.01$ and $P<0.05$, respectively.
n.s.: not significant.
Other fatty acids, i.e. C12:0, C14:0, C15:0, C17:0, C20:0, C14:1n5, C18:1n7, C20:1n9, C20:1n7, ni15, ni16:1, ni16:2, ni17:1, ni16:4, C18:3n4, C18:4n3, C20:3n3, C20:4n3, C22:5n3, C18:3n6, C20:2n6, C20:3n6, C22:4n6 and C22:5n6, in quantity lower than 0.050 g/100 g of d.m., are not reported in the table but have been included in computing the % of total lipids.

Table 4
Fatty acid percentages on total fatty acid content in the abdominal muscle

	Sex		Feeding regime (FR)		Depuration days (DD)	Sex * DD	FR * DD	r.s.d.
	♀	♂	*Ad libitum*	Restricted				
Fatty acids (% of total F.A.)								
C16:0	13.44^{b}	14.17^{a}	13.64	13.98	n.s.	n.s.	n.s.	1.01
C17:0	1.26^{b}	1.53^{a}	1.41	1.38	*	n.s.	n.s.	0.35
C18:0	7.18	7.52	7.16	7.54	n.s.	*	n.s.	0.86
ΣSFA	23.46^{b}	24.65^{a}	23.69	24.43	*	*	n.s.	1.55
C16:1n7	4.25^{A}	2.52^{B}	3.39	3.38	***	***	n.s.	0.71
C18:1n9	23.86	22.85	22.98	23.73	n.s.	*	n.s.	3.21
ΣMUFA	30.56^{A}	27.52^{B}	28.71	29.37	*	*	n.s.	3.19
$ni^{1}17:1$	1.48	1.27	1.40	1.35	n.s.	n.s.	n.s.	0.60
ΣNot identified F.A.	2.39	2.54	2.50	2.42	n.s.	**	n.s.	0.56
ΣPUFAn4	0.05	0.03	0.05	0.03	**	n.s.	n.s.	0.04
C18:3n3	3.91	3.95	4.06	3.80	n.s.	*	n.s.	1.12
C20:5n3	16.14	16.29	15.95	16.48	n.s.	n.s.	n.s.	2.59
C22:6n3	6.56^{B}	7.99^{A}	7.33	7.22	n.s.	*	n.s.	1.46
ΣPUFAn3	27.84	29.53	28.69	28.69	n.s.	n.s.	n.s.	3.96
C18:2n6cis	6.78	6.25	6.68	6.35	**	n.s.	n.s.	1.62
C20:4n6	6.80	6.99	7.25^{a}	6.54^{b}	**	n.s.	n.s.	1.19
ΣPUFAn6	15.69	15.70	16.35^{a}	15.04^{b}	n.s.	n.s.	n.s.	1.94

F.A. = fatty acids; r.s.d. = residual standard deviation
Superscripts denote significant differences (A, B: for $P<0.01$; a, b: for $P<0.05$).
***, **, and * denote $P<0.001$, $P<0.01$ and $P<0.05$, respectively.
n.s.: not significant.
The fatty acids C12:0, C14:0, C15:0, C20:0, C14:1n5 and C18:1n7, C20:1n9, C22:1n9, ni15:0, ni16:1, ni16:2, C20:3n3, C20:4n3, C22:5n3, C20:2n6, C22:5n6 in percentage lower than 1%, are not reported in the table but have been included in computing the % of total fatty acids.

4. Discussion

Our study confirms that *P. clarkii* accumulates microcystin in its organs/tissues, particularly in the intestine, and pinpoints the possibility of detoxifying crayfish abdominal muscles without losing their quality. Besides, microcystin is more abundant in small individuals and in the hepatopancreas of females.

4.1. Differences between sexes and size classes

Consistent with Vasconcelos et al.'s results (2001), we found that microcystin mostly accumulates in crayfish intestine and less in the abdominal muscle. Other organs can accumulate toxins, such as the hepatopancreas (e.g. in *Pacifastacus leniusculus*, Lirås et al., 1998, and *Cherax quadricarinatus*, Saker and Eaglesham, 1999), and the gonads (e.g. in the shrimps *Palaemon modestus* and *Macrobrachium nipponensis*, Chen and Xie, 2005; in *P. clarkii*, Chen and Xie, 2005). Contrary to finding by Chen and Xie (2005) that in 31% of cases the level of toxins in the abdomen of *P. modestus* and *M. nipponensis* is higher than the limit set by the WHO, *P. clarkii*'s abdominal muscle appears to accumulate a relatively low level of toxins, possibly due to the weak blooms of the summers 2005 (mean concentration: 1.76 μg L^{-1}) and 2006 (mean concentration: 0.20 μg L^{-1}).

A higher content of microcystin-LR was found in small rather than in large crayfish. This may be due to the juveniles being more carnivorous than the adults: they mostly prey on macroinvertebrates (Arrignon, 1996) that in their turn feed on Cyanobacteria. Risks for humans are however low because small crayfish are usually excluded from catches. Similarly, females intensify their feeding activity in October (i.e. the period of sampling) after their confinement in burrows during brood care (Huner, 1988): this might explain the higher concentration of microcystin recorded in their hepatopancreas.

4.2. Depuration

Vasconcelos et al. (2001) showed that after two weeks of feeding with toxin strain of *M. aeruginosa*, *P. clarkii* accumulates up to 2.9 μg microcystin/dry weight. During the subsequent two weeks of depuration, a fast decrease in the toxin was recorded in analogy with what recorded in mussels (Shummway, 1990; Vasconcelos, 1995). In our study, the concentration of microcystin significantly decreased with time in the abdominal muscle, but not in the intestine, independently of the sex and the feeding regime. A small and not significant increase was recorded at day 14, as found in the mussel *Mytilus galloprovincialis* (Vasconcelos, 1995; Amorim and Vasconcelos, 1999) and in *P. clarkii*'s intestine (Vasconcelos et al., 2001). This might be due to cycles in the production and degradation of protein phosphatases to which microcystin is bounded (Vasconcelos et al., 2001).

Different median values of microcystin concentration of the abdominal muscle were recorded in 2005 and 2006; however, this difference might be spurious, being the result of a diverse sample size. In fact, in 2005 the abdomens from different individuals were pooled using a total of 102 crayfish, while in 2006 for day 0 we used 8 crayfish.

4.3. Assessment of food quality

The yield and the colour of crayfish abdominal muscles are the most appreciated indicators of food quality. During depuration, the former did not change with time, as a confirmation that this highly plastic species adapts easily to the rearing conditions. Neither is the yield affected by the feeding regime, although its value (12.5%) is slightly lower than the values reported in the previous literature for this and other crayfish species (i.e. 15% in a North-American population of *P. clarkii*, Huner, 1994; 13.7% in *P. leniusculus* and 12.6% in *Astacus leptodactylus*, Harlioglu and Holdich, 2001). Various factors are known to influence crayfish meat yield, such as age, season, sex, reproductive state, weight, and mineralization of the exoskeleton (Rhodes and Holdich, 1984; Huner and Lindqvist, 1985; Huner et al., 1988; Gu et al., 1994). Yield, but not weight, does not differ between sexes, as previously found in the same species (Parisi et al., 2005) and in *C. quadricarinatus* (Thompson et al., 2004).

Some colour parameters changed with time, particularly after day 6. Indeed, β-carotene and lutein, responsible for crayfish colouration, are usually obtained from the diet (e.g. in *Cherax tenuimanus*, Sommer et al., 1991). So, in our study the use of feed without pigment supplementation may have caused an inadequate colouration (Huner and Meyers, 1979; Harpaz et al., 1998). Moreover, the diet used in this study may have affected the free water content (higher at day 21 than at day 0). The muscle ability to retain water (high water holding capacity, WHC) is an important indicator of quality. Textural and firmness properties (influencing the WHC) of *P. clarkii*'s meat change with seasons (Silva et al., 1991) but also with the feed supplied, and may depend on the confinement of crayfish during rearing.

Crayfish muscles are characterised by high levels of proteins (13.24% on dry matter) and low levels of lipids (0.71% on dry matter). Particularly abundant are fatty acids containing long-chains of n3 PUFAs, such as EPA (C20:5n3) and DHA (C22:6n3) (Rosa and Nunes, 2003; Buckup et al., 2008), which prevent cardiovascular pathologies (i.e. Kinsella 1987a,b; Harris, 1989; Sidhu, 2003; Thies et al., 2003). Lipid content found in this study was lower than the values recorded in previous studies. Wen et al. (2003) found a lipid content of around 7–8% in the whole body of *P. clarkii* fed different sources of dietary lipids and Rosa and Nunes (2003) recorded 2.7–3.4% of total lipid content (mostly phospholipids) in the abdomens of the shrimps *Aristeus antennatus* and *Parapenaeus longirostris*, and of the lobster *Nephrops norvegicus*. The most abundant fatty acids were C16:0 (among the SFA), C18:1n9 (among the MUFA), C20:5n3 (among the PUFAn3), and C18:2n6cis and C20:4n6 (among the PUFAn6). A similar profile was recorded by Rosa and Nunes (2003), but DHA (17.2–23.4%) was higher than EPA (13.8–15.5%) and the values of atherogenicity and trombogenicity indices were higher than those here obtained. Wen et al. (2003) reported a higher content of DHA in the whole body of *P. clarkii* than the one recorded in the present study.

Together with the feeding regime, sex did not influence lipid content and quality, as shown by the content and percentage of fatty acids. A similar pattern was found in both males and non-spawning females of *C. quadricarinatus* (Kong et al., 2006) and in *Parastacus defossus* (Buckup et al., 2008). Fatty acids are mostly composed of structural lipids (Rosa and Nunes, 2003), which might explain the constant content of muscle lipids regardless of sex, feeding regime, and time.

5. Conclusions

Crayfish accumulate more toxins in the intestine and less in its edible part, the abdominal muscle. The latter, but not the intestine, can be detoxified after a short period of depuration before marketing, without altering its quality and in particular its chemical composition and healthiness. Our results also pinpoint the need to adopt an appropriate diet that might reduce the negative effects recorded here on some indicators of food quality (i.e. colour and WHC). Since these effects are evident after the first week of treatment and since the decrease in microcystin is obtained at day 6, at least when blooms of Cyanobacteria are weak, we suggest a time of depuration shorter than the one tested here, which might also lead to decreased costs of the procedure. To improve the safety of crayfish meat, it is also suggested to remove the intestine prior to crayfish consumption.

Notwithstanding our encouraging results here, further studies are obviously needed to investigate the possible transfer of toxins from crayfish to other components of the community and their likely magnification along the food chain.

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